Research Article

Dulce de leche-like product enriched with emulsified pecan oil: Assessment of physicochemical characteristics, quality attributes, and shelf-life

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"Dulce de leche" (DL) is a rich, creamy, caramel-like milk-based sauce or spread, prepared by concentrating whole milk with sucrose, reaching a lipid content of about 6% w/w. A DL-like product with pre-emulsified pecan oil as fat source was developed. Nonfat milk, xanthan gum, sugars, pecan oil, and natural tocopherols were included in the formulation prepared in a pilot-plant scale and stored at room temperature. Pecan oil-DL showed physicochemical and rheological characteristics similar to those of commercial DL containing ~6% milk fat. A slight increase in viscosity was observed after 2 months of storage, remaining almost constant thereafter. Crystal formation was not observed and microbial counts were low. Slight lipid oxidation was experienced after 138 days. DL fatty acid (FA) profile showed high content of unsaturated FA (UFA, 89–90% w/w), mainly oleic and linoleic acids, effectively protected by both tocopherols and antioxidant compounds. Pecan oil-DL presented good sensorial characteristics (above 7 points on a 10 point-hedonic scale), with 82.6% less saturated FA and 200% more UFA than a traditional milk fat-product, being an alternative product with lipid profile enriched in unsaturated fatty acids.

Practical applications: The "dulce de leche" (DL) jam-like developed could be considered an alternative to the traditional DL, with better nutritional value considering its lipid composition. It has a fatty acid (FA) balance in favor of unsaturated FA as well as absence of cholesterol in the formulation because it is a product elaborated with nonfat milk. DL formulated with pecan oil showed physicochemical, rheological, and sensory characteristics similar to commercial products that contain about ~6% milk fat. It showed good stability during storage at room temperature, with no lactose crystal formation, and only slight lipid oxidation. The product was successfully developed in the laboratory and also produced in a pilot plant scale, thus, it is feasible to produce it at an industrial scale.

Keywords: Antioxidants / Dulce de leche / Pecan oil / Rheology / Unsaturated fatty acids

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1 Introduction

"Dulce de leche" (DL), a concentrated sweetened milk product obtained by heat treatment, is highly consumed in Latin America and additionally exported to other countries such as USA and Spain. DL is traditionally prepared by concentrating 3% w/v fat milk with sucrose and vanilla using heat until ~70% total soluble solids at atmospheric pressure is reached, obtaining a lipid content of about ~6% w/w. Sodium bicarbonate can also be used to increase the typical brown color and attractive flavor caused by Maillard reaction, favored at pH 6–7, and to avoid the lumps formation that result from the concentration of acidic compounds generated during evaporation [1]. One of the main causes of quality loss during storage is due to perception of sandiness because of lactose crystallization. To avoid it sucrose is usually partly replaced by other sugars (not more than 400 g/kg of total sugars should be added according to the

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Abbreviations: DL, dulce de lech; **FA,** fatty acid; **UFA,** unsaturated fatty acids

Código Alimentario Argentino [2]) so lactose crystallization is retarded by the increase in viscosity of the solution and reducing the rate of growth of lactose crystals, or by diminishing lactose content through partial enzymatic hydrolysis [3].

Dietary guidelines [4] suggest that most consumed fats should come from polyunsaturated and monounsaturated fatty acid (FA) sources, such as fish, nuts, and vegetable oils. Argentinean milk fat contains about 58% w/w of saturated fatty acids (SFA), mainly C16:0 and C14:0, with almost 30% w/w of unsaturated fatty acids (UFA), being C18:1 the main one [5], FA profile that is reflected in the consumed DL. The true effect of SFA on human health has recently come under debate and they are not considered as harmful as once believed. Trans fatty acids have been identified as an important cause of cardiovascular disease and the resulting clinical end points such as strokes and heart attacks. In addition, paradoxically, recent research has now identified an important cardioprotective role for a subcategory of trans fats, the ruminant trans fats [6]. However, it can still be concluded that, individually or as a whole, they do not have the same positive benefits as mono and especially polyunsaturated FA. The elimination, reduction, or partial substitution of animal fat in dairy foods could be a healthier alternative, but it produces, in most cases, perceptible changes in color, flavor, and especially texture [7] since fat plays multiple roles in dairy products contributing to their appearance, mouthfeel, creaminess, smoothness, cast, handling, processing, stability, and satiety [8–10]. Several authors have studied the improvement of the lipid profile in dairy products. Ye et al. [11] incorporated fish oil in processed cheese; Cunha et al. [12] replaced part of the dairy cream with vegetable fat in spreadable processed cheese. Besides inulin seems particularly suitable for fat replacement in low-fat dairy products as it may contribute to an improved mouthfeel [13]. Diverse authors showed that especially long-chain inulin addition to different low-fat dairy products resulted in enhanced creaminess [14, 15].

In addition, 2015–2020 USDA Dietary Guidelines [4, 16] consider healthy the pecan oil obtained by pressing of pecan nut (*Carya illinoensis*). Pecan oil contains no trans fats [17], has neutral flavor; it is ideal for cooking and frying at high temperatures due to its high smoke point (243°C) but, because of its high UFA content is particularly susceptible to lipid oxidation [18, 19]. However, it possesses antioxidant activity which is likely due to its high content of tocopherols and other minor components such as phospholipids [20]. If pecan oil is incorporated as UFA source to enrich dairy formulations, probably a higher concentration of antioxidants may be needed to prevent lipid oxidation during processing and storage. Nowadays consumers are putting a demand to replace synthetic antioxidants with natural alternatives [21, 22], so natural tocopherols are an adequate option.

The objectives of this work were: (i) to develop a healthier dulce de leche produced from skim milk with added pecan oil, studying the adequate oil emulsification conditions, and the effect of natural tocopherols concentration on oxidative stability; (ii) to obtain the pecan oil-DL in a pilot-scale plant and to study its physicochemical, rheological, microbiological, sensory, and other quality characteristics, analyzing possible quality changes during its storage at room temperature.

2 Materials and methods

2.1 Materials

Sterilized skim milk (maximum 0.5% w/v fat according to Código Alimentario Argentino, 1999, UHT, Sancor, Cooperativas Unidas Ltda., Santa Fe, Argentina), sucrose (food grade, Ledesma SAAI, Tucumán, Argentina), anhydrous glucose (analytical grade, Parafarm, Saporiti, Buenos Aires, Argentina), xanthan gum (Sigma–Aldrich de Argentina S.R.L., Buenos Aires, Argentina), sodium bicarbonate, and potassium sorbate (both analytical grade, Anedra, Buenos Aires, Argentina) were used for the manufacture of the products. Lactase (125 $NLUL^{-1}$, Chr. Hansen Holding A/S, Buenos Aires, Argentina) was applied to reduce lactose content of the milk. Pecan oil (NUCANA, Entre Ríos, Argentina) was employed as lipid source. Natural tocopherols (Tocomix^{TM}70, AOM S.A., Buenos Aires, Argentina, at least 63% w/w no-α tocopherols, mainly d-γ/d-β tocopherol [43.8% w/w] and d-δ tocopherol [19.3% w/w]) were added to the oil in order to control lipid oxidation in the product.

2.2 General procedure of pecan oil-DL preparation

Product formulation of dulce de leche with pecan oil contained 172.7 g sucrose, 29 g glucose, 280 mg potassium sorbate, 1 g xanthan gum, and 23 g pecan nut oil for each liter of milk used. Milk (2 L) was previously incubated at 4°C during 24 h with lactase (2.6×10^{-2} g enzyme L^{-1} milk) to reduce lactose content by 60% following Hansen's specifications, and reached a 13° of Dornic acidity (1° is equivalent to 0.1 g/L lactic acid) with sodium bicarbonate. Afterward sucrose, glucose, and potassium sorbate were dissolved in the milk. At the same time xanthan gum was dissolved in part of total milk (300 mL) at 60°C during 3 h with constant stirring, and an adequate amount of pecan oil (46 g, 5% w/w in the final product) with added tocopherols was pre-emulsified in another skim milk aliquot (300 mL).

Finally, all ingredients were mixed in a stainless steel pan (4 L capacity) with small glass beads to avoid overheating and fouling at the bottom. An electric heating plate (IKA, RCT basic, Guangzhou, China) was employed to heat the product until the desired total soluble solid content had been reached when tested with a Digit-080 refractometer (CETI Optical Instruments, Brussels, Belgium), previously

calibrated with a standard refractive index glass [23]. The final temperature was 102°C and the heating stage lasted approximately 3 h. Obtained DL were poured in previously sterilized glass jars and rapidly cooled over an ice-water bath. Two batches were produced following the same procedure.

2.3 Influence of pre-emulsification conditions on o/w microstructure

Two emulsification equipments were assayed: Ultra Turrax (UT) T25, rotor S20-25-NK-196 (IKA, Steufen, Germany) and Hand-held Homogenizer (HH) (Minipimer Braun MR 390G, Buenos Aires, Argentina); emulsification conditions were 1.5, 4, and 6 min at 11 000 rpm in both cases.

Droplet size and distribution were measured using a microscope (Leica MBLB Wetzler, Germany) equipped with a built in camera. A 40× objective lens calibrated with an objective micrometer and appropriate software (Global Lab Image 2.10) were used. Aliquots of fresh samples were observed after a 1:10 dilution with distilled water [24, 25]. Digitization was based on gray level and the diameter was estimated based on the equivalent circular diameter. Over 200 droplets from several fields and sample replicates were measured to estimate the average droplet size, for each emulsifying condition. Average Sauter diameter $D_{3,2}$ was calculated per sample as follows:

$$D_{3,2} = \frac{\sum_{i=1}^{N}(n_i d_i^3)}{\sum_{i=1}^{N}(n_i d_i^2)} \quad (1)$$

where d_i = droplet diameter, N = total number of droplets, n_i = number of droplets of d_i diameter. The corresponding variances of $D_{3,2}$ were calculated [26]; the variance is related to the polydispersity of the system. Both $D_{3,2}$ and its variance are related to the stability of emulsions when the ratio volume/surface area is important.

2.4 Product stability in accelerated test

Two levels of tocopherols (500 and 1000 mg/kg) were added to the oil before DL preparation. In addition, a DL without added antioxidants was prepared as a control. Lipid oxidation was studied by keeping the products at 37°C for 120 days and periodically quantifying the thiobarbituric acid-reactive substances (TBARS) in quadruplicate [27]. Differences between TBARS value at a selected time of storage respect to the TBARS at initial (delta) were calculated and expressed as mg malonaldehyde (MDA) kg^{-1} product dry base. In addition, it was observed if any change occurred by instability of the o/w emulsion (creaming, phase separation, etc. [24, 25]).

2.5 Pilot-plant procedure to obtain pecan oil-DL

Pecan oil-DL was obtained in a large scale at the pilot-plant of the College of Agricultural Engineering, La Plata National University; 40 L skim milk was used, with other ingredients according to formulation, and tocopherols in the selected level. Procedures to reduce lactose content with lactase, to dissolve the ingredients and pre-emulsify pecan oil were equivalent as described before, with suitable stirrers, heating vessels, and using the same conditions. A double wall stainless steel evaporator (100 L capacity) working under atmospheric pressure, with paddle agitation (20 rpm) and steam heating system (4 kg/cm pressure) (CABRIO SRL, Buenos Aires, Argentina) was used. Heating stage lasted for 3 h. The obtained product was poured in sterilized glass jars and stored at 20°C in a temperature-controlled room for 180 days. The complete procedure was performed in duplicate.

Soluble solids were determined by refractometry as described above, moisture content was analyzed [28], and water activity was measured using an AquaLab Serie 3 (Decagon Devices Inc., Pullman, Washington, USA). Process yield and lipid content were calculated considering the initial and final water content and total mass of DL obtained.

CIE-LAB color parameters (lightness, L^*, redness, a^*, and yellowness, b^*) were measured ($n = 5$) at room temperature using a Chroma Meter CR-300 (Minolta Co., Ramsey, NY, USA). Duplicate pH determinations were done with a glass electrode (Phoenix, Garbsen, Germany) on a pH meter Hach sensION + pH3 (Hach, Loveland, CO, USA), at different storage times.

2.6 Sensory analysis

At the end of the experiment, acceptability analysis of the product was conducted by 74 panelists, graduate students, and faculty members in our Institute who were experienced in sensory evaluation of foods, but received no specific training. Panelists were asked to indicate how much they liked or disliked the product on a 10-point hedonic scale (10 = like extremely; 1 = dislike extremely). This scale was chosen as it is the rating system used daily for many issues in Argentina [29] according to flavor, texture, color, and overall acceptability characteristics. Experiment was conducted in an appropriately designed and lighted room.

2.7 Lactose crystallization

Giménez et al. [30] procedure was followed to investigate crystal formation in the product during storage. Approximately 0.01 g DL was weighed on a microscope slide, slightly pressured with a coverslip and observed through an optical microscope (Leica DMLB, Wetzler, Germany) with a 10× ocular lens, 10× objective lens, and using a polarizer for better viewing.

2.8 Microbial stability

Microbial counts were done from the day after production at a 60-days interval during storage of the product according to microbial criteria for this product that take account of the coagulase-positive *Staphylococci*, molds, and yeast counts (GMC 137/96, [31]). Initial dilutions were made by aseptically blending of 10 g of sample with 90 mL of peptone solution (1 g/L) in a Stomacher blender (West Sussex, UK) for 60 s at normal speed. Appropriate serial dilutions were pour plated in YGC agar (Merck KGaA, Darmstadt, Germany) for mould and yeast counts (incubated at 30°C for 5 days), and spread plated in Baird Parker Agar (Biokar, Beauvais, France) for coagulase-positive *Staphylococci* (incubated at 35–37°C for 24–48 h).

2.9 Rheology

Rheological measurements were carried out using a controlled stress rheometer (Haake RS600; ThermoGap, Karlsruhe, Germany) provided with a serrated parallel plate fixture (35 mm diameter, 1 mm gap), at 20.0 ± 0.1°C controlled by means of a controlled fluid bath unit and an external thermostatic bath. After positioning the sample on the sensor system, it was allowed to rest for 10 min before starting the corresponding measurement. To minimize dehydration, samples were covered with a thin layer of silicone oil and a solvent trap was used. Four replicates were performed for each sample from the day after production at a 60-day interval during storage.

2.9.1 Steady shear flow

To obtain steady-state flow curves (viscosity, η, vs. shear rate, $\dot{\gamma}$), the shear stress was measured by increasing the shear rate in steps from 0.01 to 100 s^{-1}. The maximum measuring time per point was set at 300 s, but all measurements were obtained before the cut-off time, so it could be assumed that steady state was almost attained.

Herschel–Bulkley's model [32] was used to model steady-state flow curves:

$$\sigma = \sigma_0 + K\dot{\gamma}^n \quad (2)$$

where K is the consistency coefficient, n is the flow behavior index, σ_0 is the yield stress, and the shear rate.

2.9.2 Oscillatory shear tests

Dynamic rheological measurements (storage modulus, G' and loss modulus, G'', vs. frequency, ω) were done using a frequency range from 0.01 to 100 s^{-1}. Linear viscoelasticity region was determined through stress sweep tests at a fixed frequency (6.28 rad/s).

The software used to fit the rheological models was IRIS Rheo-hub (Amherst, MA, USA).

2.10 Lipid oxidation and fatty acid profile

TBARS values expressed as mg of malonaldehyde (MDA) kg^{-1} product were determined to evaluate the lipid oxidation in the DL at different storage times.

Lipids of the products were extracted by Röse-Gottlieb method (24105) [33] at initial, 90, and 180 days of storage. FA profiles were determinate by gas chromatography (Hewlett Packard 6890, GA, USA) equipped with fused silica capillary column (Chrompack CP-SIL 88, length 50 m, ID 0.25 mm, 0.1 μm film, Varian Inc., Palo Alto, CA, USA) and flame-ionization detector at PROPIA laboratory (Universidad Nacional de La Plata). FAME (Fatty acid methyl esters) were identified by comparing their retention times with those of commercial standard FAME (NuCheck Prep. Inc., Elysian, USA). Peak areas were determined and calculated as normalized area percentages of FA [34, 35].

2.11 Statistical analyses

Analyses of variance were conducted separately on the dependent variables analyzed. For simultaneous pairwise comparisons, least significance differences (LSD) test was chosen. Differences in means and F-tests were considered significant when $P < 0.05$. All statistical procedures were computed using the SYSTAT software (SYSTAT, Inc., Evanston, IL, USA).

3 Results and discussion

3.1 Oil pre-emulsification methodology

Figure 1 shows mean Sauter diameter ($D_{3,2}$) obtained analyzing oil droplet sizes for the different emulsifying conditions tested. The smaller $D_{3,2}$ values were obtained for the emulsification made with a hand-held homogenizer during 4 min ($D_{3,2} = 130$ μm). So, this condition was selected for the oil pre-emulsification in order to obtain the most stable emulsion.

3.2 Lipid oxidative stability in accelerated assays

Figure 2 presents the obtained results for lipid oxidation of pecan oil-DL with different tocopherols levels in an accelerated assay at 37°C, expressed as the increment in TBARS with respect to their initial value (ΔTBARS, mg MDA kg^{-1} dry product). The three formulations showed a significant increase ($P < 0.05$) in TBARS during storage. The tocopherols mix used, mainly a mixture of D-β, D-γ y D-δ tocopherols, produced a significant inhibition ($P < 0.05$) of lipid oxidation in the product, making a contrast with the

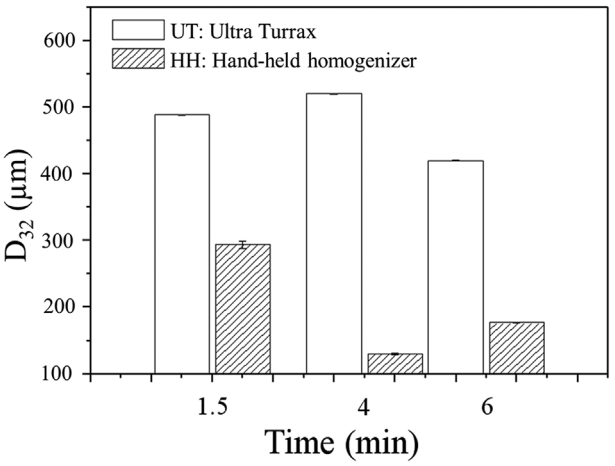

Figure 1. Sauter mean diameter ($D_{3,2}$) corresponding to different emulsifying conditions tested. UT, ultra turrax; HH, hand-held homogenizer. Vertical bars show standard error of the mean.

work of Tenorio [36] who did not found a protection with α-tocopherols (0–500 ppm) in dairy systems enriched with UFA. As the heating of the mix proceeds in order to obtain DL, Maillard reaction between milk proteins and short chain carbohydrates takes place. Thus, the presence of some Maillard compounds could be associated with antioxidant effects [37–43] and contribute with tocopherols to the inhibition. TBARS levels reached values between 5.8 and 6.8 mg MDA kg^{-1} product after 120 days of storage.

Besides, pecan oil-DL samples presented no visual signs of instability during storage as no oil phase separation was detected. Based on these results, 1000 mg/kg of tocopherols was selected as an appropriate level to add to the product, particularly considering the long heating time required above 100°C, which contributed to start oxidative reactions.

3.3 Pecan oil-DL obtained at a pilot-scale

The product with pre-emulsified pecan oil produced at the pilot-plant presented 65 °Brix of soluble solids content, 33.6 (0.3)% w/w moisture content, and a water activity of 0.845 (0.003), while color parameters were 38.7 (1.2), 8.9 (0.1), and 19.4 (0.6) for L^*, a^*, and b^*, respectively. These results were in the range of commercial products [28]. The process yield was 30.4%. According to the formulation and yield, the lipid content of pecan-DL was 6.56% w/w, within the range corresponding to Argentinean [28] and Brazilian [44] commercial DL.

Sensory analysis results indicated that more than 90% of panelists assigned more than seven points to all the attributes, showing a good acceptability of the product, despite a large dispersion among consumer preferences as has been informed by Gaze et al. [45]. The obtained average scores were: 8.5 (0.1), 8.7 (0.1), 9.1 (0.1), and 8.4 (0.2), for general acceptability, texture, color, and taste, respectively.

3.4 Storage of pecan oil-DL at 20°C

Visual inspections of the products showed that they remained stable after 6 months of storage, with no oil phase separation. Moreover, in microscopic observations no tomahawk-like shape crystals, typical of α-lactose, were detected [46], avoiding a typical defect that occurs in DL. This was probably due to a ~60% of reduction in the lactose content of

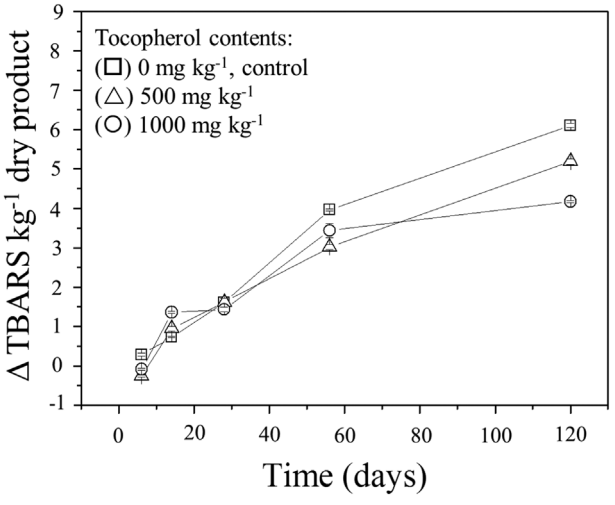

Figure 2. Lipid oxidation (ΔTBARS, mg MDA/kg dry product) of "dulce de leche" with pecan oil and natural tocopherols stored at 37°C. Tocopherol contents: 0 mg/kg, control (□), 500 mg/kg (△), and 1000 mg/kg (◯). Vertical bars show standard error of the mean.

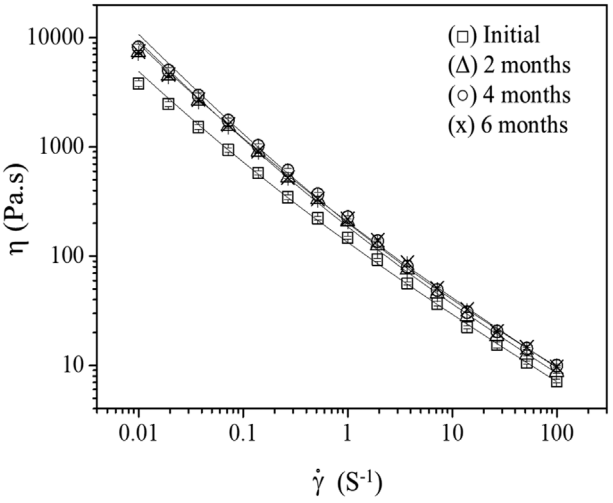

Figure 3. Effect of storage time at 20°C on flow curves of "dulce de leche" with pecan oil, produced in the pilot-scale plant. Initial (□), 2 (△), 4 (○), and 6 (x) months.

Table 1. Changes in Herschel–Bulkley (HB) parameters and plateau moduli (G_N^0) of pilot-plant dulce de leche with pecan oil during storage at 20°C*

	HB model			
Storage time (months)	σ_0 (Pa)	K (Pa · s^n)	n	Plateau modulus G_N^0 (Pa)
0	34.5^{c} (4.1)	99.0^{b} (4.2)	0.42^{b} (0.01)	2.5×10^{3a} (227)
2	77.9^{ab} (3.0)	107.4^{b} (0.7)	0.43^{ab} (0.01)	3.4×10^{3ab} (358)
4	95.0^{a} (8.9)	106.1^{b} (5.5)	0.46^{a} (0.02)	4.0×10^{3b} (528)
6	65.8^{b} (5.1)	138.2^{a} (2.5)	0.40^{b} (0.01)	4.0×10^{3b} (578)

*Standard errors of the mean are given between parentheses. Different superscripts within the same column indicate significant differences ($P < 0.05$). $N = 4$.

milk after lactase activity. Moulds and yeast, and coagulase-positive Staphylococci counts were lower than the detection limits of the methods (2 log CFU g^{-1}), showing that the thermal treatment and aseptic packaging were adequate to obtain a 6 month shelf-life.

3.5 Rheological characteristics

Figure 3 shows that pecan oil-DL obtained in the pilot-plant presented the typical pseudoplastic flow behavior of DL prepared with regular milk [47, 48], and within the ranges of commercial DL [28]. It is important to note that DL consistency varies from one brand to another, since the formulations usually include different hydrocolloid levels alone or in combination, in addition to their different content of solids [28, 44, 45, 48]. In the first 2 months of storage, there was a small increase in apparent viscosity, keeping it constant afterwards.

Herschel–Bulkleýs model adequately fitted flow curves at different storage times (Fig. 3). Yield stress, σ_0, strongly increased after 2 months, remaining constant thereafter, while consistency coefficient, K, increased between 4th and 6th month at 20°C (Table 1). Besides, storage time did not affect the flow consistency index, n (Table 1). This increase in apparent viscosity was in concordance with that observed in low-fat DL formulations [49]. Thus the manufactured DL exhibited age thickening due to structural rearrangements. Structure development is thought to involve not only casein micelles but also the whey proteins (especially if these have suffered a high degree of denaturation during thermal processing) and the fat globules. These changes could be associated with several modifications in the continuous phase (mainly hydration of milk proteins, carbohydrates, and xanthan gum) that could happen during the storage and would increase the volume fraction of dispersed components. Some or all of the following structural modifications may take place: Casein aggregation [50]; hydrocolloids or hydrocolloid/milk components interactions [51]; milk proteins gelation [52]; glucose polymerization [53]; xanthan gum aggregation [54, 55], and association of denaturated

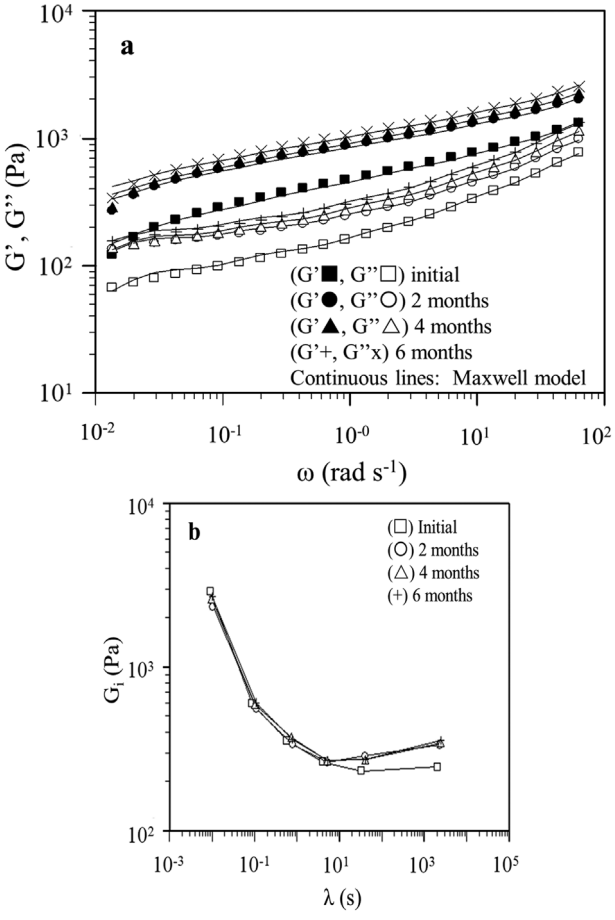

Figure 4. A. Frequency dependence of storage (G') and loss (G'') moduli of dulce de leche with pecan oil obtained in the pilot-scale plant. Storage time at 20°C: initial (G' ■, G'' □), 2 months (G' ●, G'' ○), 4 months (G' ▲, G'' △), 6 months (G' X, G'' +). Continuous lines represent Maxwell model fitted to experimental data. B. Discrete relaxation times spectrum for "dulce de leche" with pecan oil obtained in the pilot-scale plant, corresponding to different storage times at 2°C. Initial (□), 2 (○), 4 (△), and 6 (+) months.

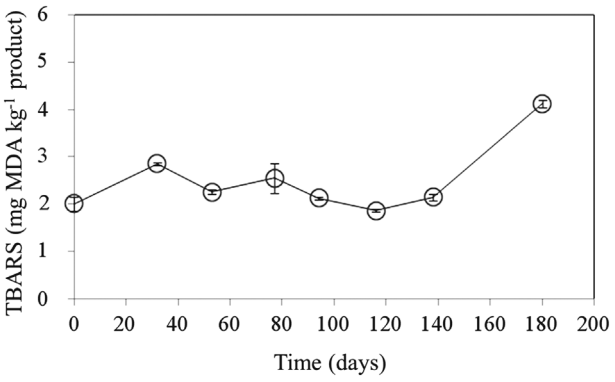

Figure 5. TBARS evolution of "dulce de leche" with pre-emulsified pecan oil and 1000 mg/kg of tocopherols obtained in the pilot-scale plant during the storage at 20°C. Vertical bars show standard error of the mean.

β-lactoglobulin and casein micelles [56]. According to Rovedo et al. [48], it could take up to two months at 20°C for DL proteins to reach an equilibrium configuration, agreeing with the results obtained in this work.

Pecan oil-DL mechanical spectrum showed a slightly dependence of G' and G'' with frequency at every storage time, without a minimum in G''. Additionally, a 6-element Maxwell model [57] satisfactorily fitted experimental results (Fig. 4a). A similar behavior has been previously reported by Ranalli et al. [49], saving some small differences depending on the DL type.

According to Maxwell, storage and loss moduli could be related to the discrete relaxation spectrum by:

$$G'(\omega) = G_e + \sum_{i=1}^{N} G_i \frac{(\omega\lambda_i)^2}{1+(\omega\lambda_i)^2} \quad (3)$$

$$G''(\omega) = \sum_{i=1}^{N} G_i \frac{(\omega\lambda_i)}{1+(\omega\lambda_i)^2} \quad (4)$$

where G_i corresponds to the elastic response of each spring and λ_i are the relaxation times of each Maxwell element. A non-linear iterative numerical method was used to determine the parameters N, G_i, and λ_i. The computed G_i and λ_i values were used to predict the storage and loss moduli.

As can be observed in Fig. 4a, there is an excellent agreement between experimental and predicted values, confirming the accuracy of the calculations.

Discrete relaxation spectra obtained for pecan-DL during storage is presented in Fig. 4b. Spectrum shapes showed two phases, one at short time periods (10^{-2}–10 s), and the other, at longer time periods (10–10^3 s), passing through a minimum where storage moduli were flat. More noticeable differences with storage time were observed in this last phase (Fig. 4b).

The plateau moduli G_N^0 (Table 1) were evaluated from the discrete relaxation spectra at each storage time as: $G_N^0 = \sum(G_i)$. G_N^0 reflects the molecular architecture of the polymers and it is proportional to the number of entanglements per unit volume and inversely proportional to the average molecular weight of the molecular segment between entanglements. It is a measure of the contribution of the entanglements between polymeric molecules to the elastic or storage modulus [58]. An increase in G_N^0 between initial and 2 months of storage was observed, reflecting the structural rearrangements that occurred during those first months in agreement with the increase in viscosity previously described.

Table 2. Fatty acid profiles of pecan oil "dulce de leche" stored at 20°C*, as well as of the pecan oil used, and Argentine milk fat

	Pecan oil-DL				
Fatty acid (g/100 g)	Initial	3 months	6 months	Pecan oil	Milk fat**
C14:0	0.2[a]	0.1[b]	0.1[b]	0.1[b]	10.20
C16:0	6.6	6.4	6.4	6.5	24.10
C18:0	3.05[a]	3.1[a]	2.7[b]	3.0[a]	10.71
C18:1 n-9	62.5	62.3	62.4	62.3	25.78
C18:2 n-6	25.4	24.8	26.0	25.3	1.68
C18:3 n-3	1.0[b]	1.3[a]	1.2[ab]	1.2[ab]	0.93
SFA	10.6	9.9	9.8		
UFA	89.4	90.1	90.1		
SFA/UFA	0.1	0.1	0.1		
AI	0.08	0.08	0.08		
TI	0.2	0.2	0.2		

*Different superscripts within the same row indicate significant differences ($P < 0.05$). $N = 2$. SFA, saturated fatty acids; UFS, unsaturated fatty acids; AI, atherogenicity index; TI, thrombogenicity index.
**From Rebechi et al. [5].

3.6 Lipid stability and FA profile

Figure 5 shows TBARS evolution of the products stored at 20°C. Up to 138 days, TBARS levels remained constant, showing an increase afterwards. Again, TBARS levels reached in DL with pecan oil were lower than others reported by Candebat et al. [59] for condensed milk (65.8–84.9 mg MDA kg^{-1} after 5 month of storage at 28°C), and were not associated with sensorial changes in the product.

Table 2 shows fatty acid profiles obtained in the lipid phase of the product at different storage time. As it was expected a high UFA level (89–90% w/w), mainly oleic (C18:1 n-9 cis), and linoleic (C18:2 n-6 cis) acids was found, in accordance with their proportion in the pecan oil, and a lower level of SFA, in opposition to FA profile of milk fat. Therefore, the product presented a lower SFA/UFA ratio with respect to the traditional product obtained with whole milk. In spite of the high UFA content and its high oxidation susceptibility, UFA levels did not show significant differences ($P > 0.05$) throughout storage, reflecting, again, the antioxidant effect of the added tocopherols and other natural compounds of the product.

Since scientific evidence that the fat type is more important than the total amount ingested for the risk of cardiovascular disease, predictive equations as atherogenicity (AI) and thrombogenicity (TI) indexes have been used to estimate the FA profile effect over plasmatic cholesterol and lipoprotein levels [34]. Values higher than 1 are associated to a detrimental effect over human health [60]. It could be seen (Table 2) that the obtained indexes were well below 1, which indicates a beneficial effect of the product FA profile.

Taken into account Argentinean milk fat and pecan oil FA profiles (Table 2), it could be concluded that 100 g of pecan oil-DL would have 82.6% lower SFA and 200% higher UFA contents with respect to a traditional product.

4 Conclusions

Although milk fat was replaced by pre-emulsified pecan oil, the obtained DL-like product had appropriate rheological properties, similar to the traditional product. Quality attributes of pecan oil-DL remained stable during 6 months at 20°C and the product showed high acceptance by untrained panelists. The product was successfully developed in the laboratory and in a pilot-scale plant, which indicates that it is feasible to produce it on an industrial scale. The use of vegetable oils in the formulation resulted in a product similar to "dulce de leche" with a lipid profile enriched in unsaturated fatty acids.

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